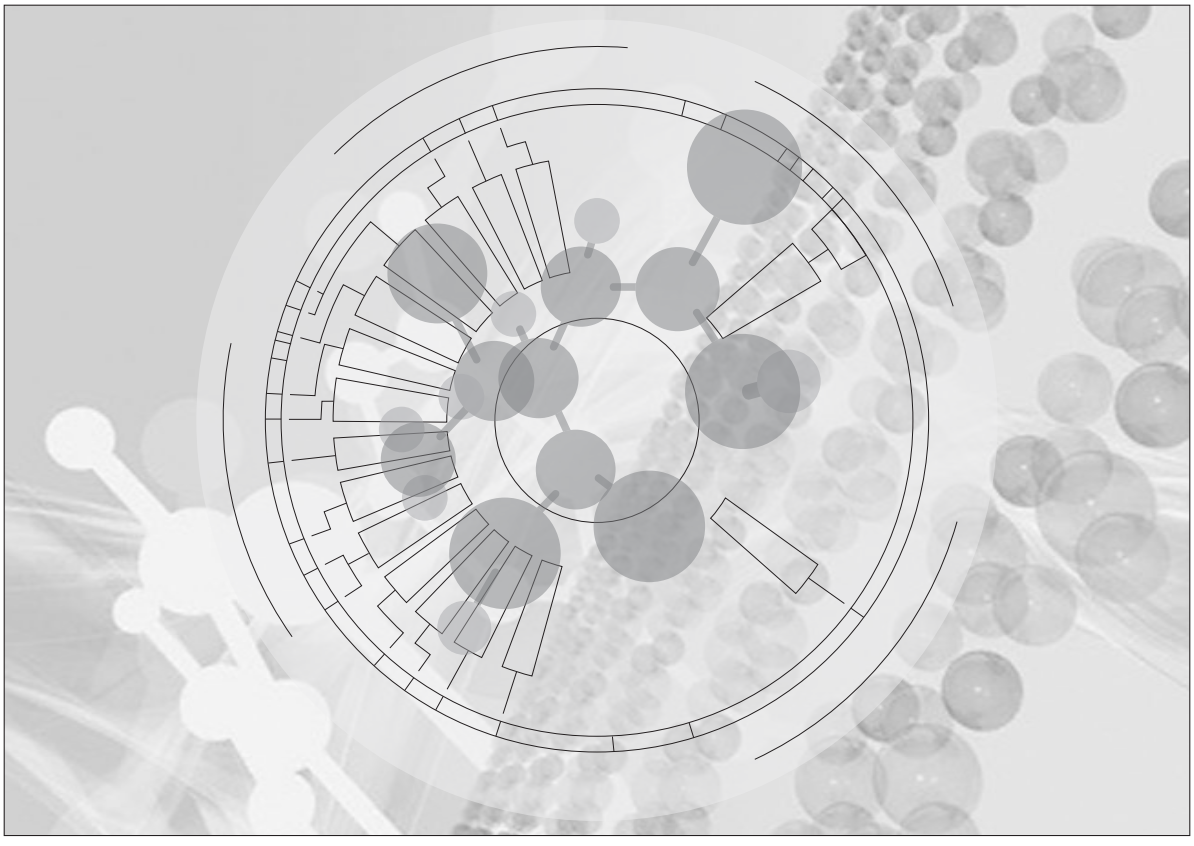

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The Role of Extracellular Matrix in the Aetiology of Gestational Hypertension and Preeclampsia: A Preliminary Study

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ABSTRACT

The aim of this study is to determine the alteration of extracellular matrix (ECM), namely fibronectin and collagen type IV, in the aetiology of hypertensive disorders of pregnancy. Gestational hypertension and preeclampsia account for 5-7% of the maternal mortality and morbidity worldwide. Gestational hypertension and preeclampsia is characterised by blood pressure of 140/90 mmHg or greater after the 20th week of gestation. The aetiology of gestational hypertension and preeclampsia is closely related to the disorder of placenta implantation. It is thought that shallow trophoblast invasion into maternal decidua causes malfunction of the utero-placental arteries, thus leading to both the diseases. The exact cause of shallow trophoblast invasion remains controversial. Placenta implantation involves activation and migration of trophoblast into the decidua and myometrium. These processes are dependent on extracellular matrix, where activation of appropriate adhesion molecules and integrins are essential for appropriate trophoblast activity. In this study, collagen type IV and fibronectin were investigated in a total of 30 placentas by immunohistochemistry and H&E stains. Observation on the staining intensity of the antibodies were done in both villous (foetal) and decidual (maternal) areas. Statistical analysis was performed using Mann-Whitney test. The analysis showed a significant increase of number of villi in gestational hypertensive and preeclamptic placentas compared to normotensive placentas. Staining intensity of collagen type IV and fibronectin suggested an alteration of level of these components in gestational hypertension and preeclampsia group compared to normotensive group and this alteration may play a role in the aetiology of the two hypertensive diseases.

Key words: Placenta, preeclampsia, collagen type IV, fibronectin, immunohistochemistry

INTRODUCTION

Hypertension in pregnancy is classified into 4 different categories, which are preeclampsia, gestational hypertension (which is also known as pregnancy induced hypertension-PIH), chronic hypertension and preeclampsia superimposed on chronic hypertension (Caren and Seely, 2006). In this study, the focus was on Gestational Hypertension (GH) and preeclampsia (PE) (Gifford and Cunningham, 2000).

In patients with GH and preeclampsia, the blood pressure criteria taken on two consecutive occasions with at least 4 h apart, are the same. The only difference between preeclampsia and GH is that in preeclampsia is the presence and absence of proteinuria (= 300 mg in a 24 h urine collection), respectively (Caren and Seely, 2006).

The GH (5-9%) and preeclampsia (5-7%) remain one of the leading causes of maternal mortality in the world, including Malaysia (Zhang *et al.*, 1997).

In a normal pregnancy, implantation of the human placenta occurs when the trophoblast cells migrate and invade the maternal uterine wall. The invasion extends through the entire decidua up to the myometrium (Burrows *et al.*, 1996). Without a proper utero-placental artery system, the blood flow to the placenta and foetus are compromised and this ischaemic situation will lead to preeclampsia and GH (Zhou *et al.*, 1997).

There are several hypothesis that have been proposed to explain the reason for the inadequate invasion of trophoblast that leads to preeclampsia and GH. Genbacev *et al.* (1997) suggested that low oxygen levels (5-8%) that was found in foetal-maternal interface favours trophoblast proliferation and invasion but not under hypoxic conditions (< 2%) where the trophoblast will behave just like in the condition of preeclampsia (Debra and Simcha, 2007). There is another theory proposed in recent years stating that preeclampsia is a disease of nulliparous woman. The maternal immune response towards paternal antigens (semen) and thus cross react with trophoblast were thought to be the reason for poor trophoblast invasion and placental development (Genbacev *et al.*, 1997). And this explains the high incidence of preeclampsia in teenagers (Peters *et al.*, 2004).

All these processes are dependent on the extracellular matrix (ECM) where activation of appropriate adhesion molecules and integrins are essential for appropriate trophoblast activity (Debra and Simcha, 2007). Besides that, in order to achieve successful invasion, the trophoblast must be able to degrade certain ECM by inducing the genes involved in digestion of ECM (Goldman and Yagel, 2002; Lim *et al.*, 1997).

Different tissues have different cells that contribute to the formation and maintenance of the extracellular matrix (Mosher *et al.*, 1992). In the normal development of placenta, in order for the invasion of the foetal trophoblast into maternal uterine wall to occur, maternal tissue has to be degraded (Denker, 1993). This involves degradation of structural proteins of extracellular matrix, which includes collagen type IV and fibronectin (Risteli *et al.*, 1984; Kliman and Feinberg, 1990; Feinberg *et al.*, 1991). Immunohistochemical staining on oncofetal fibronectin showed intense staining present at foetal membrane-decidual region (Lockwood *et al.*, 1991; Guller *et al.*, 2003) suggesting that oncofetal type of fibronectin plays a crucial role in mediating cell adhesion and attachment at materno-foetal junction. In preeclampsia and GH, it was shown that soluble form of cellular fibronectin is elevated in preeclamptic woman compared to normotensive woman, even before the manifestation of the clinical diseases (Taylor *et al.*, 1991).

The objective of this study is to determine the alteration of extracellular matrix (ECM), namely fibronectin and collagen type IV, in the aetiology of GH and preeclampsia. Our hypothesis being that the alteration of certain specific elements in the extracellular matrix that are functionally related to the invasiveness of the cytotrophoblast and the vascularisation materno-foetal interface of the placenta could not lead to the development of preeclampsia and other hypertensive diseases of pregnancy.

MATERIALS AND METHODS

Patients: This study was conducted from placental tissue obtained from 30 patients at the Department of Obstetrics of the teaching hospital of our Medical University, from 2008 to 2009, after informed consent and approval from the Ethical committee of our University. In this study, our target population was pregnant women with high blood pressure (higher than 140 mmHg systolic and 90 mmHg diastolic) after the 20th week of gestation. Those patients who had this symptom were categorized into 2 different groups, namely GH and PE, depending whether there was any proteinuria present during the course of pregnancy.

The placenta was weighed, cleaned and inspected. A small portion (2×2×2 cm) of placenta, with the entire thickness, was cut out using surgical blade on a random site on the cotyledon. The placenta was then being washed in normal saline to remove any blood clots. It was then submerged into 10% neutral buffered formalin at room temperature. After fixation of the placenta tissues, the tissue was processed before being embedded into paraffin wax. After the placenta tissues were blocked in paraffin wax, they were sectioned (at 4 μm thickness) using a Leica RM 2135 Rotary Microtome.

Staining procedure: Tris-Buffered Saline (DAKO) was prepared by dissolving one full sachet (16.15 g±10%) into 1 L of distilled water and mixed in a Schott and Duran bottle. Twenty microlitter of TBS was pipette out into a coplin jar to ensure that an ideal pH of 7.6 based on the literature provided by DAKO (Mettler Toledo 320).

Target Retrieval Solution (DAKO) was used on paraffin-embedded sections mounted on sialinised slides for heat-induced target retrieval before immunohistochemistry staining, at a dilution ratio of 1:10 with pH 9.0.

Mouse Monoclonal (TB-21) (DAKO) Antibody to Transforming Growth Factor-β was diluted at a ratio of 3:1000 based on the literature provided by DAKO, with Antibody Diluent with background reducing components.

Substrate chromogen (DAKO) used at a dilution of 1:50. For this experiment, 5 mL of Dual Link System-HRP (DAB+) chromogen was diluted into 250 mL of DAB+ chromogen buffer.

The tissue was embedded in paraffin wax with ceresin using L-moulds. Sections of 4 μm were cut from the paraffin blocks. This was done accurately using a rotary microtome knife (Leica RM2135). The cut sections were placed on the sialinised slide from DAKO and dried at 35°C for 5 min before staining.

Hematoxylin and eosin staining: Before staining, the tissues were dewaxed using hot air oven at 60 °C for 45 min. Next, the slides were taken through xylene and decreasing strengths of alcohol till distilled water. The slides were then stained with and then differentiated in acid-alcohol, immersed in bicarbonate solution to 'blue' them and stained with Eosin Y. Slides were mounted with cover slips using DPX Vecta Mount Mounting Medium.

Immunohistochemistry staining: Target retrieval was done using Target Retrieval Solution (TRS) solution at 95°C for 45 min. The slides were brought to the adequate pH of 7.6 by placing them in TBS for 10 min.

Next, Dual Endogenous Enzyme Block was then carefully applied to the tissues on the slides for 20 min, to prevent excess background staining by other tissue proteins. The primary antibody was then applied in amounts that were sufficient to cover the entire tissue on the slides. The

primary antibody was left on the tissue for 90 min at room temperature. Next, Labeled Polymer HRP was applied carefully on the tissues for 40 min. Next, the slides were rinsed in TBS again and immersed for 10 min.

Substrate chromogen, was applied on the tissue at room temperature for 20 min. After that, the slides were counter-stained in Meyer's Haematoxylin for 10 min, to allow for better, enhanced visualization of nuclei of cells. The slides were then rinsed under tap water, blued, dried and mounted with coverslips using DPX.

The slides were observed for the expression of collagen IV and fibronectin in the placental tissues using a Nikon brightfield light microscope. Images were captured with a 5.1 megapixel Evolution MP digital camera. Image Pro Express Software was used to process the images. The images were then analysed.

Analysis of sections: The slides were observed for morphological changes between normotensive, gestational hypertensive and preeclamptic placentas. NIS Elements for Basic Research software was used for photo capturing and analysis of result.

Eight randomly selected fields were taken at 100x magnification in each slide. In each selected field view, numbers of complete villi were counted in a specific area using a calibrated grid by NIS Elements software.

Analysis of collagen and fibronectin expression by immunohistochemical staining: The slides were observed for the expression of Collagen type IV and fibronectin in the placenta using a NIS Elements for Basic Research software. Images were captured with camera attached to the software. The images were then analysed.

Cells that express Collagen type IV and Fibronectin positivity was characterised as a brownish bronze coloured pigmentation within the cytoplasm. Slides with positive staining were categorised as (+), (++) or (+++) depending on the intensity of the staining. Slides with negative staining for Collagen type IV and Fibronectin were labelled as 0. Observation on the staining intensity of the antibodies were done in both villous (foetal) and decidual (maternal) areas.

Statistical analysis: Statistical Analysis was performed using Statistical Package for Social Science (SPSS), version 16.0. For analysis of H and E slides between the normotensive group with gestational hypertensive group and between normotensive group with preeclamptic group, comparisons between mean value of total villi count were done. Independent T test was the parametric test used to examine significance. Data was analysed at 95% confidence interval and a p-value of less than 0.05 was considered significant. For analysis of IHC slides (both collagen type IV and fibronectin) between normotensive group with gestational hypertensive group and between normotensive group with preeclamptic group, comparison between mean grade were done. Mann-Whitney test was the non-parametric test used to examine significance. Data was analysed at 95% confidence interval and a p-value of less than 0.05 was considered significant.

RESULTS

Under microscope, histological morphology of gestational hypertensive placentas and preeclamptic placentas were observed and compared with normotensive placentas. The most obvious difference between the hypertensive placentas and normotensive placentas is that there are marked increased in the amount of villi in hypertensive placentas compared to the pre eclampsia placentas (Fig. 1a, b) and was much lower in normotensive placentas.

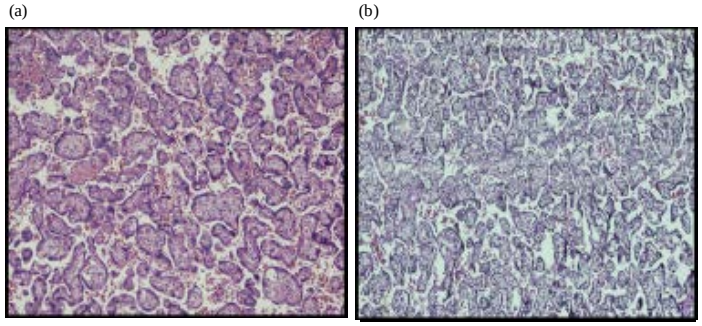

Fig. 1: Difference between total number of villi In pre eclampsia Placenta and Gestational Hypertensive Placenta showing the overcrowding of villi in gestational (b) hypertensive placenta compared to (a) Preeclampsia placenta. Microscopic magnification at 100x

Table 1: Grading of collagen type IV and fibronectin expression intensity

Grade	Percentage of cells in tissue expressing collagen type IV and fibronectin
0	0% of the cells expressed collagen type IV and fibronectin
+	Less than 33% of the cells expressed collagen type IV and fibronectin
++	33-66% of the cells expressed collagen type IV and fibronectin
+++	More than 66% of the cells expressed collagen type IV and fibronectin

With the aid of a calibrated grid, we counted the total number of villi presented in a randomly selected area. Based on the date obtained (Table 2), normotensive placentas (n = 10) have the lowest number of villi count among the three groups, with the mean value of 91.85 units of villi in the fixed area. For gestational hypertensive placentas (n = 10), it was higher at 105.28 units. Preeclamptic placentas have the highest number of villi in a fixed area among all three groups, with the mean value of 110.53.

The result of the independent t-test showed a significant increase ($p<0.05$) in the number of villi between normotensive placentas and gestational hypertensive (Table 3). There was also a statistically significant ($p<0.01$) increase between normotensive placentas and preeclamptic placentas (Table 4).

Comparison of collagen type IV immunohistochemical staining between normotensive, gestational hypertensive and preeclamptic placentas.

Under the light microscope, the intensity of Collagen Type IV staining was compared between normotensive, gestational hypertensive and preeclamptic placentas. They were graded as 0, +, ++, +++ based on the positivity of their staining. Observation was done in both the villous and the decidual parts (Table 1).

Based on the data obtained, there is a slight increase in staining intensity in villi from normotensive placentas to gestational hypertensive and preeclamptic placentas. When the Mann-Whitney test is applied, it is seen that the increase in the intensity from normotensive placentas to gestational hypertensive placentas is not statistically significant (Table 5). However, the increase in staining intensity from normotensive placentas to preeclamptic placentas is statistically significant (Table 6).

Comparison of fibronectin immunohistochemical staining between normotensive, gestational hypertensive and preeclamptic placentas.

Table 2: Mean total number of villi in normotensive, gestational hypertensive and preeclamptic placentas

	Mean total villi count of the 8 random areas		
No.	Normotensive	Gestatioual hypertensive	Preeclamptic
1	99.00	108.75	118.50
2	108.50	90.00	125.25
3	86.00	113.75	115.25
4	123.25	112.50	92.75
5	96.50	107.25	118.75
6	88.00	104.50	103.25
7	85.00	102.50	122.50
8	82.50	128.75	91.50
9	78.75	103.75	106.25
10	71.00	81.00	111.50

Table 3: SPSS Output of Independent t-test showing that there is a significant increase in total number of villi between normotensive placentas and gestational hypertensive placentas

	Levene's test for equality of variances		t-test for equality of means						
								95% confidence interval of the difference	
	F	Sig.	t-value	df	Sig. (2-tailed)	Mean difference	SE difference	Lower	Upper
No. of villi									
Equal variances assumed	0.587	0.453	-2.108	18.0	0.049	-13.42500	6.36829	-26.80427	-0.04573
Equal variances not assumed			-2.100	17.5	0.050	-13.42500	6.36829	-26.83035	-0.01965

Table 4: SPSS Output of Independent t-test showing that there is a significant increase in total number of villi between normotensive placentas and preeclamptic placentas

	Levene's test for equality of variances		t-test for equality of means						
								95% confidence interval of the difference	
	F	Sig.	t-value	df	Sig. (2-tailed)	Mean difference	SE difference	Lower	Upper
No. of villi									
Equal variances assumed	0.460	0.506	-3.047	18.00	0.007	-18.70000	6.13686	-31.59307	-5.80693
Equal variances not assumed			-3.00	16.907	0.007	-18.70000	6.13686	-31.65308	-5.74692

Under light microscope, intensity of fibronectin staining was compared between normotensive, gestational hypertensive and preeclamptic placentas. They were graded as 0, +, ++, +++ based on the positivity of their staining. Observation was done in both the villous and decidual parts.

Based on the data obtained, there was no staining in the villi at all for all three groups of the placentas and hence they were all graded 0. No statistical analysis was done on this data.

In the decidua, there is a decrease in the intensity of fibronectin staining from normotensive placentas to gestational hypertensive and preeclamptic placentas. Mann-Whitney test showed that the decrease in the intensity from normotensive placentas to gestational hypertensive placentas is not statistically significant. However, the decrease in staining intensity from normotensive placentas to preeclamptic placentas is statistically significant.

Table 5: SPSS output of Mann-Whitney Test showing the increase in the staining intensity in villi from normotensive placenta to gestational hypertensive placentas are statistically insignificant

Test statistics	Grading	Grouping	N	Mean rank	Sum of ranks
Mann-Whitney U	33.500	Grading normal	10	8.85	88.50
Wilcoxon W	88.500	GH	10	12.15	121.50
Z	-1.407	Total	20		
Asymp. Sig. (2-tailed)	0.159				
Exact Sig. [2×(1-tailed Sig.)]	0.218				

Table 6: SPSS output of Mann-Whitney Test showing the increase in the staining intensity in villi from normotensive placentas to preeclamptic placentas are statistically significant

Test statistics	Grading	Grouping	N	Mean rank	Sum of ranks
Mann-Whitney U	25.000	Normal	10	8.00	80.00
Wilcoxon W	80.000	PE	10	13.00	130.00
Z	-2.190	Total	20		
Asymp. Sig. (2-tailed)	0.028				
Exact Sig. [2×(1-tailed Sig.)]	0.063				

DISCUSSION

Of a total of 30 placental specimens that were collected, 10 specimens were from normotensive women, 10 were from gestational hypertensive women and 10 were from preeclamptic women. A larger sample size was impossible due to time constraints, as well as limited number of cases available in the Hospital Tuanku Ja'afar. It was reported that only about 6% of the total pregnant women that were admitted into hospital in Malaysia were gestational hypertensive and preeclamptic patients. Moreover, due to certain misconceptions and/or certain cultural practices, many patients were not willing to donate their placenta for research purposes. In addition to that, some deliveries happened in odd hours, where placental tissue sample collection by the team was impossible. Therefore, the numbers of target patients were well below the 6% of the total obstetric cases mentioned earlier. Given all the difficulties in patient recruitment, the total number of placental specimens (n = 30) collected in this pilot study was reasonable and statistically adequate.

The H and E staining of all the placenta sections revealed obvious histopathological changes (if any) in all three groups. We are particularly interested in the difference of total number of villi count between normotensive placentas and both the hypertensive diseased placentas. This is because in a normotensive placenta, trophoblast cells in the villi could influence the invasion of villi into maternal decidua and modification of maternal vasculature. With that, foetal blood vessels within each villous could form materno-foetal blood flow that could facilitate nutrient and gaseous exchange.

The mean numbers of villi count in gestational hypertensive placentas and preeclamptic placentas are significantly higher than those in normotensive placentas. This result corresponds with research done by Arnholdt *et al.* (1991). Arnholdt *et al.* (1991) suggested that there was an increase in the proliferation rate of the trophoblast in preeclampsia. The reason behind this increase of number of villi in diseased placentas could be that hypertensive placentas might be trying to increase the total surface area of nutrient and gas exchanging sites (by increasing the number of villi) to compensate the hypoxic state of the placenta due to the lack of trophoblastic invasion into the maternal decidua. However, there have been no conclusive studies that could explain the mechanism behind the increased of villi proliferation. The increase in the number of villi

(which in turn increase the mass and cellularity of placenta) also corresponds to reports that have observed that the placenta is relatively bigger and heavier in preeclampsia (Fox, 1988).

Immunohistochemistry staining collagen type IV: Patchy brown stainings were seen in both villi and decidua area of all three groups of placenta specimens. This shows that collagen type IV is present in both the areas. As we have discussed earlier, collagen type IV was found abundantly in the basement membrane. The presence of a distinct collagen type IV networks is important for the intrinsic cohesiveness of the basement membrane when they are under mechanical stress (Poschl *et al.*, 2004). Collagen type IV is also involved in the formation of a functional and stable basement membrane (Poschl *et al.*, 2004).

In present study, the staining intensity of collagen type IV in villi increases significantly in preeclamptic placentas compared to normotensive placentas. However, the increase in intensity seen in gestational hypertensive placentas as compared to normotensive placentas is statistically insignificant. Nevertheless, there is an increasing trend in staining intensity seen from normotensive placentas to both diseased placentas. This finding is very interesting because it was in contrast to a report by Risteli *et al.* (1984), which states that the level of collagen type IV in the gestational hypertensive and preeclamptic placental villi is unchanged compared to the normotensive placenta. Since then, there have been no other studies that have documented any concrete finding on this matter. Therefore, the results of this study which is in contradiction to the earlier study certainly warrants a deeper investigation involving a larger population in order to have a better understanding on the aetiology of gestational hypertension and preeclampsia with regards to collagen type IV in villi, which seems to have been largely ignored until now.

In the decidua, the increase of collagen type IV (as evidenced by the increase in staining intensity of both normotensive placentas to gestational hypertensive and preeclamptic placentas) are statistically significant. This shows that in both the diseased state, the collagen type IV present in the maternal decidua is abnormally high. This may be an important finding as we know that preeclampsia occurs when the invasion of trophoblast into decidua is disturbed (Naicker *et al.*, 2003). Since, collagen type IV was found only in basement membrane, the increase in staining intensity would indicate that the basement membranes in decidua are highly thickened compared to decidua in normotensive placentas. This thickened basement membrane may hinder the invasiveness of trophoblast by matrix metalloproteinase (MMP) thus leading to gestational hypertension and preeclampsia.

Immunohistochemistry staining-fibronectin: The fibronectin examined in this study has a unique glycopeptide domain in type III segment (IIICS), called oncofetal fibronectin (onfFN) class (Feinberg *et al.*, 1991). It is found specifically in human tumors and pregnancy tissues, as the name have suggests. Under the normal light microscope, brown immunohistochemical staining of oncofetal fibronectin is present only in the maternal deciduas and not in the floating villi. This finding is consistent throughout all the three groups of placentas. This would suggest that fibronectin is present and secreted only in the maternal decidua and invading villi, but not in the floating vilh. A similar phenomenon was reported by Feinberg *et al.* (1991) stating that oncofoetal fibronectin is present only in the attachment zone of the placento-uterine junction. Therefore both placental villi (floating villi) and uterine tissue (non-implantation site) are deprived of fibronectin (Feinberg *et al.*, 1991).

As shown in our study, negative staining of fibronectin was observed in villi throughout all 30 placentas. In the decidua, the staining intensity of fibronectin decreased significantly in preeclamptic placentas as compared normotensive placentas, but the decrease in intensity in gestational hypertensive placentas compared to normotensive placentas were statistically insignificant. Nevertheless, there is a decreasing trend in staining intensity seen in both diseased placentas compared to normotensive placentas. Since fibronectin is secreted by invading villi (Guller *et al*., 2003), this finding would suggest that the number of invading villi is lower in both the diseased groups of placentas compared to the normal groups of placentas. This result relates to the fact that the invasion of trophoblast into maternal decidua is inadequate in gestational hypertension and preeclampsia (Naicker *et al*., 2003). Therefore, we would be inclined to conclude that fibronectin plays an important role in the migration and invasion of invading trophoblast. Any alteration of its expression or concentration might lead to serious pathological disease state.

There have been no studies done on levels of oncofoetal fibronectin concentration in placental tissues. However, other studies have suggest that foetal fibronectin is elevated in maternal plasma as well as amniotic fluid in preeclamptic patients (Taylor *et al*., 1991; Kupfermine *et al*., 1995). These findings suggest that there is either an increase in the production of foetal fibronectin from chorionic trophoblast, or there is an increased in the leakage of foetal fibronectin into maternal circulation and amniotic fluid due to abnormal interaction between chorionic trophoblast and decidua in preeclamptic placenta. The results of this study might support the leakage theory as there is a decreased in fibronectin concentration level in preeclamptic placental tissues.

The results of immunohistochemical staining of collagen type IV and fibronectin clearly demonstrates that in gestational hypertension and preeclampsia, there is an alteration of concentrations of certain extracellular matrix elements. We believed that the increase in collagen type IV in diseased placentas could be hindering the degradation process of the thickened basement membrane thus leading to limited trophoblastic invasion. On the other hand, the decrease in fibronectin in diseased placentas could also directly hamper the invasion process of trophoblast as fibronectin is proven to help in the migration of trophoblastic cell.

The main limitation of this study was that all the placenta samples that were collected were at term. Any changes of the concentration of collagen type IV and fibronectin could be the cause or an effect of limited invasion of trophoblast into the maternal decidua. In other words, it would be impossible to state whether these alterations of extracellular matrix components caused gestational hypertension and preeclampsia to happen, or if they were the effect of the disease itself. Conversely, increased collagen type IV in the maternal decidua in diseased placentas may be due to the lack of degradation of this extracellular matrix component by matrix metalloproteinase due to some other factors. On the other hand, decrease in fibronectin could just be the effect of low numbers of invading villi. Nevertheless, the data obtained in this could still be important information because these two extracellular matrix components can serve as good indicator of gestational hypertension and preeclampsia diseases although their usefulness is limited in identifying the disease in their early stages.

To overcome this problem, placentas that are aborted around the first or second trimester should also be used. This would actually help us see the process and stages of invasion of the trophoblast and also determine the location, concentration and function of collagen type IV and fibronectin more efficiently. Time and budget constraints did not did not allow this to be done in this study. As mentioned earlier, even getting term placentas was difficult in such a short period and hence getting aborted diseased placentas would be almost impossible under the given circumstances.

CONCLUSIONS

From the findings of this preliminary study, it can be concluded that when compared to normotensive group, the gestational hypertension and preeclampsia group had significantly increased amounts of collagen type IV in the maternal decidua, which may hinder the invasiveness of trophoblast. The significant decrease of fibronectin in the maternal decidua may decrease the migration and invasion of the trophoblast.

Future studies in an extended, multicentric setting would certainly help throw more light on these elusive and evasive diseases that continue to puzzle and mystify us the conditions that we know as hypertensive disease of pregnancy.

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